Appendix C: Unmarked (clean) amended claim listing.

We Claim:

1. (Amended) A mutually exclusive folding domain molecular switch comprising a fusion protein that includes at least one ubiquitin insert protein having an insert domain comprising a regulatory domain of ubiquitin lying between an amino terminal and a carboxyl terminal of said at least one insert protein, said insert domain being associated with a first quantity of free energy; and, a barnase target protein having at least one surface loop that begins at an alpha carbon of a first surface loop amino acid and terminates at an alpha carbon of a second surface loop amino acid, said at least one surface loop comprising a cytotoxic target domain of barnase of said target protein, said target domain being associated with a second quantity of free energy, wherein, said at least one insert protein is inserted at a point within said at least one surface loop between said alpha carbon of said first surface loop amino acid and said alpha carbon of said second surface loop amino acid, such that an amino-carboxyl length extending between an alpha carbon of said amino terminal of said at least one insert protein and an alpha carbon of said carboxyl terminal of said at least one insert protein is at least two-times greater than an alpha carbon-alpha carbon length extending between said alpha carbon of said first surface loop amino acid and said alpha carbon of said second surface loop amino acid.

2 (Original) The fusion protein of claim 1, wherein said insert domain exists in either a folded or unfolded conformation and said target domain exists in either a folded or unfolded conformation, said insert domain and said target domain comprising a

cooperative and reversible conformational equilibrium such that if said insert domain is in its folded conformation, said at least one target domain is in its unfolded conformation and vice versa.

3. (Original) The fusion protein of claim 2, wherein all or part of said first quantity of free energy is made available to drive a folding of said target domain from its unfolded conformation by means of a first controllable effector signal, and all or part of said second quantity of free energy is made available to drive a folding of said insert domain from its unfolded conformation by means of a second controllable effector signal

4. (Original) The fusion protein of claim 3, wherein said first controllable effector signal is selected from the group comprising ligand binding, pH, temperature, chemical denaturants, or mutations in either said insert domain or said target domain.

5. (Original) The fusion protein of claim 3, wherein said second controllable effector signal is selected from the group comprising ligand binding, pH, temperature, chemical denaturants, or mutations in either said insert domain or said target domain.

6. (Original) The fusion protein of claim 2, wherein said insert domain and said target domain are disenabled from simultaneously co-existing in their respective folded conformations.

7. (Canceled) The fusion protein of claim 2, wherein said insert domain and said target domain are disenabled from simultaneously co-existing in their respective unfolded conformations.

8. (Original) The fusion protein of claim 2, wherein any excess of said first quantity of free energy of said insert domain that is not necessary to stabilize said insert domain in its folded conformation is spontaneously transferred, through the structure of said fusion protein, to said target domain to unfold it from its folded conformation.

9. (Original) The fusion protein of claim 2, wherein any excess of said second quantity of free energy of said target domain that is not necessary to stabilize said target domain in its folded conformation is spontaneously transferred, through the structure of said fusion protein, to said insert domain to unfold it from its folded conformation.

10. (Original) The fusion protein of claim 2, wherein said at least one insert protein comprises human ubiquitin, said insert domain comprises a regulatory domain of human ubiquitin, said target protein comprises barnase, said at least one target domain comprises a cytotoxic domain of barnase, said amino-carboxyl length is about 38 Å, said first surface loop amino acid comprises proline in the number 64 position ("Pro64"), said second surface loop amino acid comprises threonine in the number 70 position ("Thr70"), and said alpha-carbon-alpha-carbon length is about 10.4 Å.

11. (Original) The fusion protein of claim 10 wherein said regulatory domain of human ubiquitin and said cytotoxic domain of barnase comprise a cooperative and reversible conformational equilibrium, that may be determined by said controllable first and second effector signals.

12. (Canceled) A method for the production of a protein comprising the steps of:

a. selecting a linker containing first and second restriction sites between a Lys66 and a Ser67 codon of a barnase gene;

b. using said first and second restriction sites of said linker to operationally insert a ubiquitin gene between two amino-acid codons of said linker, thereby creating a ubiquitin-barnase fusion gene;

c. fully sequencing said ubiquitin-barnase fusion gene to verify its integrity;

d. using enzymes to operationally insert said ubiquitin-barnase fusion gene into any plasmid of a BL21 (DE3) family, thereby creating an interim ubiquitin-barnase fusion expression plasmid;

e. operationally inserting a gene for barstar and its natural promoter from *Bacillus amyloliquifaciens* into said interim ubiquitin-barnase fusion expression plasmid, thereby creating a ubiquitin-barnase fusion-barstar complex plasmid;

f. cloning said gene for barstar into a T7 promoter-containing plasmid conferring resistance to an antibiotic other than ampicillin onto cells transformed by said T7 promoter-containing plasmid, thereby creating a barstar plasmid;

g. transforming *E. coli BL21* (DE3) cells grown at about 20 to 37 degrees C in any medium compatible with E. coli growth using both said barstar plasmid and said ubiquitin-barnase fusion-barstar complex plasmid, and inducing said *E. coli* BL21 (DE3) cells with about 100 mg/L isopropyl b-D-thiogalactopyranoside;

h. harvesting said transformed *E. coli* cells by centrifugation after about 2 to 12 hours; after said induction;

i. placing said harvested *E. coli* cells in10 mM sodium phosphate at a pH of 7.5, thereby creating a solution of harvested *E. coli* cells;

j. lysing said solution of harvested *E. coli* cells by repeated freeze-thaw cycles in the presence of about 10mg/liter lysozyme, thereby creating a lysate;

k. adding about 10 mg/liter DNase I to reduce the viscosity of said lysate;

l. centrifuging said reduced viscosity lysate to remove insolubles, thereby forming a supernatant;

m. adding about 8 M urea to said supernatant to dissociate bound barstar;

n. Removing said dissociated barstar from said supernatant by passing said supernatant through an anion exchange chromatography resin to yield a solution;

o. loading said solution onto a cation exchange column;

p. washing said solution with about 10 mM sodium phosphate (pH about 7.5) and about 6 M urea;

q. eluting said solution using a 0 to 0.2 M NaCl gradient;

r. Removing said urea from said dilution by dialysis against double-distilled water to yield barnase-ubiquitin fusion protein.